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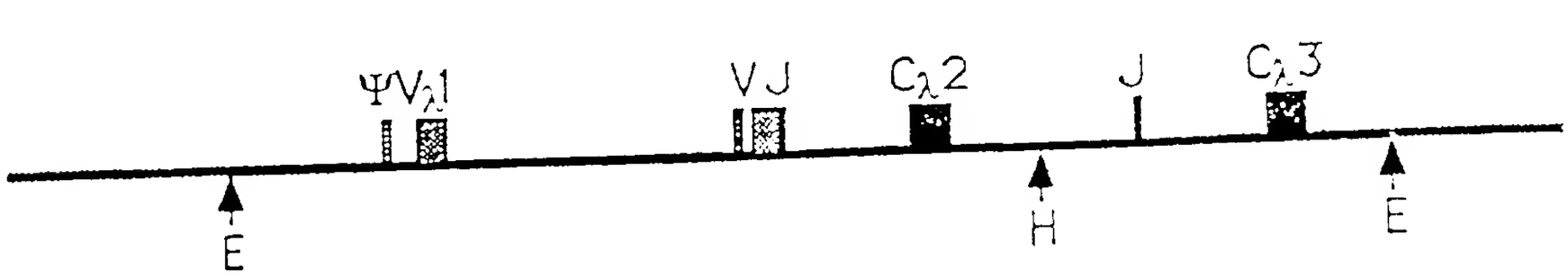


(57) Abstract

A transgenic FVB/N mouse bearing a transgene comprising a DNA sequence encoding an immunoglobulin light chain endogenous to a non-mouse animal.

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# B CELL DEFICIENT TRANSGENIC FVB/N MICE

## Background of the Invention

The field of the invention is transgenic animals.

B cells, which derive from hematopoietic precursor cells in the bone marrow, are the immunoglobulin-producing cells of the immune system. Efforts to study B cell development have been hindered by the extreme heterogeneity of the B cell population. This heterogeneity results from the fact that a mature B cell can produce any one of the approximately $10^{11}$ different antibody molecules that it is theoretically possible to generate by such DNA-level mechanisms as genomic rearrangement, imprecise joining and somatic mutation. Because B cells arise from pluripotent hematopoietic progenitor cells and proceed through a complicated maturation process that is influenced by a variety of factors both before and after interaction of the cell with antigen, the B cell population present in an animal is developmentally heterogeneous as well. There are a number of myeloma, hybridoma and pre-B cell lines that homogeneously produce a particular antibody molecule and allow study of some aspects of B cell function. However, many of these cell lines are locked at a particular developmental stage and cannot be used to study B cell development. More recently, transgenic technology has allowed researchers to create mice in which a large portion of the B cells express a particular rearranged $\kappa$ or $\lambda$ (light chain) transgene or $\mu$ (heavy chain) transgene. In general, these rearranged $\kappa$, $\lambda$ and $\mu$ transgenes, when fused to immunoglobulin gene regulatory sequences, are expressed only in lymphoid tissues of the mouse. Such transgenic mice can provide model systems for studying various aspects of immune system function, including allelic exclusion, isotype exclusion, somatic

mutation, immunoglobulin gene regulation, and interactions between immune system cells.

For example, mice bearing $\mu$ or $\lambda$ transgenes have been used to study allelic exclusion, the process by which a given B cell becomes committed to utilizing only one of the two homologous chromosomal loci for each of the heavy and light chain genes. Mice harboring a rearranged murine $\kappa$ transgene have very low levels of endogenous $\kappa$ gene expression (Storb et al. Nature 310:238, 1984) and exhibit far less rearrangement of the endogenous $\kappa$ gene loci than do their wild-type littermates (Ritchie et al., Nature 312:517, 1984; Rusconi et al., Nature 314:330, 1985). Mice with a human $\mu$ transgene fail to express murine IgM on those B cells expressing the transgene (Nussenweig et al., Science 236:816, 1987).

Mice harboring a murine $\lambda$ transgene have been used to study isotype exclusion, the process whereby each B cell in a given animal becomes committed to expressing one light chain isotype (either $\kappa$ or $\lambda$) rather than the other. Mice with a transgenic murine $\lambda$ gene driven by the murine heavy chain enhancer express the transgene in a lymphoid-specific manner, have surface $\lambda$ chain present on B cells, have sharply decreased levels of $\kappa$ gene rearrangement, and have far more $\lambda^+$ B cells than $\kappa^+$ B cells (Neuberger et al., Nature 338:350, 1989; Hagman et al., J. Exp. Med. 169:1911, 1989). This last observation is in marked contrast to the dominance of $\kappa^+$ B cells in wild-type mice. In addition to the above phenotype, Hagman et al. observed a "slight decrease in the number of $IgM^+$ B cells in the spleens of transgenic mice of all ages". Neuberger et al. noted that "the transgene causes an overall depression of B cell development as the number of spleen cells in the transgenic mice was ~70% that of their non-transgenic siblings". For these $\lambda$ transgene

experiments, a heavy chain enhancer was used to drive expression of the λ transgene because rearranged λ transgenes without added enhancer were found not to be expressed (Hagman et al. supra).

## Summary of the Invention

In general, the invention features a transgenic FVB/N mouse bearing a transgene which includes a DNA sequence encoding an immunoglobulin light chain endogenous to a non-mouse animal. In a preferred embodiment, the sequence encoding the immunoglobulin light chain gene is obtained from an animal (most preferably a human) in which the proportion of rearranged immunoglobulin light chain genes that are λ light chain genes is higher than the proportion typically found in FVB/N mice (e.g., greater than ~5% of the rearranged immunoglobulin light chain genes are λ light chain genes). Examples of such animals include, besides humans, chickens, dogs, cats, cows, horses, sheep and mink. A rearranged light chain gene is a light chain gene that has undergone recombination to bring together a region encoding a constant domain and a region encoding a variable domain, and thus is capable of encoding a complete λ light chain. Preferably, the immunoglobulin light chain constant region of the rearranged gene is chosen from the human λ1, λ2, λ3, and λ7 constant regions. More preferably, the constant region is the human λ2 constant region. Most preferably, the immunoglobulin light chain is that encoded by the U266λ gene.

Preferably, the transgene also includes an enhancer region capable of directing high level expression of the transgene, i.e., increasing the level of transcription of the gene or genes which are part of the transgene. Such an enhancer region can be, for

example, that of an immunoglobulin heavy chain gene, e.g., a mouse immunoglobulin heavy chain gene, or a portion thereof (e.g., all or part of the 995 XbaI to EcoRI subfragment) capable of directing high level expression of the transgene. The enhancer may be located upstream of the coding sequence, within an exon, or, more preferably, downstream of the coding sequence. Such enhancer regions can be individually tested for their ability to direct high level expression of a transgene by transfecting the enhancer-transgene construction into tissue culture cells as described herein. The transgenic FVB/N mice of the invention are more susceptible (preferably, significantly so) to infectious disease (such as diseases caused by Sendai virus, EDIM virus, mouse hepatitis virus, mycoplasma pulmonis, pinworm, and mites) than is a typical wild-type FVB/N mouse, a condition which at least in part is due to low B cell levels; preferably, the proportion of the transgenic animal's splenic lymphocytes that are mature B cells is four-fold lower than the proportion that are mature B cells in a typical wild-type inbred FVB/N mouse. For example, in a wild-type inbred FVB/N mouse, the proportion of splenic lymphocytes that are mature B cells is about 40%, while the proportion found in a transgenic FVB/N mouse that is one embodiment of the invention is about 2%. By "mature B cell" is meant a lymphocyte bearing surface $\mu$ heavy chain protein, whether alone or as part of an intact immunoglobulin molecule. Preferably, the transgenic FVB/N of the invention mice have at least three times as high of a percentage of splenic lymphocytes which bear detectable levels of the Sca-1 surface antigen as a typical wild-type FVB/N mouse has (which is ~10%). By "splenic lymphocytes" is meant white blood cells isolated from an animal's spleen.

Preferably, less than 4% of the splenic lymphocytes of the transgenic mice bear an amount of the B220 (Ly-5) surface antigen that is detectable in an assay such as that described below. This is in contrast to wild-type inbred FVB/N mice, in which approximately 40% of the splenic lymphocytes bear detectable B220 (Ly-5) antigen, a B cell marker.

Most preferably, the transgenic FVB/N mouse is a transgenic mouse derived from (i.e., resulting from embryological maturation and development of) an embryo deposited on August 28, 1990 with the American Type Culture Collection, Rockville, MD as ATCC No. 72003, or a transgenic descendent of such a transgenic mouse.

The invention also features a hematopoietic cell derived from a transgenic mouse of the invention.

A "transgene" is defined as a piece of DNA which is inserted by artifice into a cell and becomes a part of the genome of the animal which develops from that cell. Such a transgene may be partly or entirely heterologous to the transgenic animal. A "transgenic mouse" is a mouse having cells that contain a transgene, which transgene was introduced into the mouse, or an ancestor of the mouse, at an embryonic stage. An "enhancer region" is defined as a cis-acting DNA sequence capable of increasing transcription from a promoter that is located either upstream or downstream of the enhancer region. Such DNA sequences are well known to those skilled in the art of eukaryotic gene expression. By "hematopoietic cell" is meant a blood cell whether developmentally immature or mature, or a precursor of a blood cell. An "FVB/N mouse" is a mouse that is either a inbred mouse of the FVB/N strain or a hybrid mouse that has at least half of its genome contributed by mice of the inbred FVB/N strain. Preferred FVB/N mice of the invention are fifth (or higher) generation descendents of

founder mice generated from FVB/N embryos. These subsequent generations result from backcrossing with inbred FVB/N mice; thus the mice of each succeeding generation are, except for the transgene, more genotypically identical to inbred FVB/N mice. By "U266λ gene" is meant the 8.5 kb EcoRI/HinDIII fragment of the rearranged λ gene isolated from the human myeloma U266 cell line. By "U266λ protein" is meant the immunoglobulin light chain protein encoded by the U266λ gene.

The invention provides a useful animal model for severe B cell deficiency, characterized by low levels of functional B cells and high susceptibility to infectious disease.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## Description of the Preferred Embodiments

The drawings are first described.

### Drawings

Fig. 1 is a diagrammatic representation of a region of a plasmid bearing the human U266λ gene.

Fig. 2 is a representation of the nucleic acid sequence of a portion of the human U266λ gene.

Fig. 3 is a diagrammatic representation of the λ construct.

Fig. 4 is a representation of the nucleic acid sequence of the 995 bp XbaI fragment of pTAR-7 which contains the mouse heavy chain enhancer.

Fig. 5 is a diagrammatic representation of the λEμ construct.

Fig. 6 is a representation of the results of an RNAse protection analysis.

Fig. 7 is a representation of the nucleic acid sequence of the 9071 bp EcoRI fragment of the λEμ construct. Fig. 8 is a schematic representation of the results of an assay for cells bearing the Sca-1 surface antigen.

Production of Transgenic Mice

The transgenic mice of the invention are produced by inserting a cloned non-mouse immunoglobulin gene into the genome of an inbred FVB/N x inbred FVB/N oocyte (or an inbred-FVB/N X CD-1 fertilized oocyte or a fertilized oocyte produced from the mating of a mouse of any suitable strain and an inbred FVB/N mouse) and backcrossing the transgenic offspring with wild-type FVB/N mice so that the transgene is in an essentially pure FVB/N genetic background.

The production of several strains of transgenic FVB/N mice is described below. Two of the strains harbor the human U266λ gene fused to the murine heavy chain enhancer. Three of the strains harbor the human U266λ gene without the enhancer.

The steps involved in producing and analyzing the transgenic mice of the invention are outlined below, including construction of plasmids bearing the U266λ gene, transient transfection of these plasmids into tissue culture cells (as an initial test for cellular expression of the U266λ gene), assays for expression in these tissue culture cells, introduction of the U266λ gene into the mouse genome, and methods for analyzing the transgenic mice.

Construction of Plasmids

DNA from the IgE-λ human myeloma U266 cell line (Nilsson et al., Clin. Exp. Immunol. 7:477, 1970) was digested with EcoRI, and used to prepare a phage library (Maniatis et al., Molecular Cloning: A Laboratory Manual,

Cold Spring Harbor Laboratory, 1982). The clone containing the active λ gene (a portion of which is depicted Fig. 1; E denotes an EcoRI site, H denotes a HinDIII site) was identified by probing the library with a radiolabelled BamHI fragment of the human λ2 gene (Hieter et al., Nature 294:536, 1981). The complete DNA sequences of the human Cλ complex and the U266λ gene are available in the EMBL Data Library under accession numbers X51755 and X51754, respectively.

Two constructions were prepared: λ (which includes the U266λ gene) and λEμ (which includes the U266λ gene and a downstream murine immunoglobulin heavy chain enhancer). The λ construction was prepared by subcloning the 8.5 kb EcoRI to HinDIII fragment (the "U266λ gene", the sequence of this fragment is depicted in Fig. 2) of clone c35I containing the $V_\lambda$ pseudogene, $\Psi V_\lambda 1$, as well as the active λ gene rearranged into λ2, into EcoRI- and HinDIII-digested pBR327 to create pC36I (Fig. 3 is a diagramatic representation of a portion of this plasmid. E denotes an EcoRI site, and H denotes a HinDIII site) . The λEμ construct was prepared by cutting the λ construct at the unique HinDIII site located 0.9 kb downstream of $C_\lambda 2$. The ends were made blunt with DNA polymerase (Klenow fragment), and were then ligated to a 995 bp XbaI fragment (Fig. 4) of pTAR-7 (Banerji et al., Cell 33:727, 1983) containing the mouse immunoglobulin heavy chain enhancer (Ephrussi et al., Science 227:134, 1985) that had been similarly treated. The resulting plasmid, pc40I, is depicted in Fig. 5 (E denotes a EcoRI site. h/x denotes the XbaI/HinDIII junction). Cloning procedures were conventional techniques described in Maniatis et al. (supra).

Transfection of Human λ Gene into Tissue Culture Cells

The λ and λEμ constructs were tested for their ability to express human λ mRNA in transiently

transfected tissue culture cells. The cells used for transient expression of the human λ gene were Ly65, an IgM-κ producing human Burkitt's lymphoma cell line (Lenoir et al., Nature 298:474, 1982) and J558L, a λ producing mouse myeloma cell line (Oi et al., Proc. Natl. Acad. Sci. USA 80:825, 1983). Actively growing cells were harvested and placed in an ice-cold electroporation cuvette at $4x10^7$ cells/ml with 100 μg/ml of purified λ DNA or λEμ DNA. Electroporation was performed as described by Potter et al. (Proc. Natl. Acad. Sci. USA 81:7161, 1984) using an ISCO 494 power supply set at 2000 V with the current limited to 0.9 mA. After electroporation, the cells were left on ice for 10 minutes, and then transferred to 30 ml of growth medium. Cells were cultured for 36 hours prior to harvesting for RNA analysis by means of a ribonuclease (RNase) protection assay.

The template for production of RNase protection probes was made by inserting the 735 bp SstI to StuI fragment of the λ construct into SstI- and SmaI-digested pSP64 (Promega Biotec, Madison, WI) to generate plasmid c109I. When linearized with EcoRI and transcribed by SP6 polymerase, c109I produces a 783 nucleotide antisense RNA.

RNase protection assays were performed essentially as described by Melton et al. (Nucl. Acids Res. 12:7035, 1984). Total RNA was isolated as described by Chirgwin et al. (Biochemistry 18:5294, 1979). Uniformly labeled antisense RNA probes were synthesized by the method of Melton et al. (supra), using EcoRI-digested C109I. Probe fragments ($5x10^4$ cpm, specific activity ~$6x10^8$ dpm/μg) were hybridized to 10 μg of total RNA for 4-12 hours in the presence of 80% formamide at 50°C, according to the procedure of Berk et al. (Cell 12:721, 1977). The hybridizations were terminated by the addition of RNase A

at 40μg/ml and RNAse T1 at 2μg/ml. Samples were incubated for 10 minutes at room temperature, digested with proteinase K, phenol extracted, ethanol-precipitated, and analyzed on 0.4 mm thick, 8M urea 5% acrylamide gels as described by Melton et al. (supra). RNA was quantified by laser densitometry analysis (Molecular Dynamics, Sunnyvale, CA) of autoradiograph films.

As shown in Fig. 6, transfection with the λ construction resulted in weak expression of human λ mRNA in ('Probe' indicates the position of the probe. 'Vλ' indicates the position of the λ mRNA). Ly65 cells and slightly stronger expression in J558L cells. Transfection with the λEμ construction led to 130-fold higher expression of the human λ mRNA in Ly65 cells and 470-fold higher expression in J558L cells, compared to the level of expression of the λ construct in each such cell type. Production of Transgenic Mice Containing λ or λEμ Transgene

Transgenic mice harboring λEμ were produced as follows. The λEμ construct was digested with EcoRI, and the 9071 bp fragment containing λ2 and part of the heavy chain enhancer (Fig. 7) was purified by electroelution following gel electrophoresis (Maniatis et al., supra). The fragments were injected, as described by Muller et al. (Cell 54:105, 1988), into the pronuclei of fertilized oocytes derived from inbred FVB/N X CD-1 mice (EG) or inbred FVB/N mice (all other lines). FVB/N (Taconic Farms, Germantown, NY) is an inbred strain which is albino (c), agouti (A), H-$2^q$, and has the b alleles of Lyt-1, Lyt-2 and Lyt-3. CD-1 (Taconic Farms, Germantown, NY) is an outbred albino strain. The injected eggs were transferred to pseudo-pregnant females as described by Muller et al. (supra) and allowed to develop to term. Mice were housed in an environmentally-controlled

facility maintained on a 12 hour dark/12 hour light cycle.

Transgenic mice harboring the λ construct were produced in a similar manner using the EcoRI/HinDIII fragment of the λ construct (which contains the U266λ gene without an added enhancer).

Analysis of Transgenic Mice

At four weeks of age, each pup born from an injected oocyte was tested for presence of the appropriate transgene by Southern blot hybridization. The DNA for analysis was extracted from a section of tail (approximately 1cm) by the method described by Davis et al. (Methods in Enzymology, Grossman et al., eds., 65:404, 1980). The resulting nucleic acid pellet was dissolved in 100 μl of 10mM TRIS (pH 8.0), 1mM EDTA. 3 μl of the tail DNA preparation were digested to completion with EcoRI, electrophoresed through 1% agarose gels, and transferred to nylon filters, as described by Southern (J. Mol. Biol. 98:503, 1975). $^{32}$p-labelled probe was prepared by Klenow random priming (Feinberg et al, Anal. Biochem. 137:266, 1984) of a 1.2 kb BamHI fragment of the λ construct. Filters were incubated overnight with ~$10^8$ cpm of probe in 10 ml of buffer with 10% dextran sulfate and 50% formamide, and rinsed two times in 0.1X SSC at 25°C and two times in 0.1X SSC at 68°C. (Southern, supra) The filters were then analyzed by autoradiography.

The Southern hybridizations indicated that each of three λ founder mice, designated TG.huλ-NT, TG.huλ-NU and TG.huλ-NV had each retained the injected λ gene in multiple copies integrated at a single autosomal site. Similarly, two λEμ founder mice, designated TG.λEμ-EG and TG.λEμ-EH, had each retained the injected λEμ gene in multiple copies integrated at a single autosomal site, with TG.λEμ-EG mice harboring more of copies of the

transgene than TG.λEμ-EH mice. Each founder mouse was mated to a wild-type inbred FVB/N mouse, and the DNA of the resulting offspring was analyzed by Southern hybridization as described above. In every case the transgene was transmitted through the germline in a Mendelian fashion.

The founder animals were crossed with inbred wild-type FVB/N mice for at least seven generations; thus, the mice used in all subsequent experiments were, except for the transgene, genotypically essentially pure FVB/N mice. Backcrossed descendents of the TG.λEμ-EG founder animal are referred to as EG mice when they are heterozygous for the transgene and EG/EG when they are homozygous for the transgene. Backcrossed descendents of the TG.λEμ-EH founder animal are referred to as EH mice when they are heterozygous for the transgene and EH/EH when they are homozygous for the transgene.

The transgenic mice harboring the λEμ construct are maintained by crossing male EG and EH mice with inbred +/+ FVB/N females. As homozygous EG/EG mice do not live beyond weaning, hemizygous mice (i.e. EG and EH mice) were used for each of the experiments described below.

Mice of each transgenic strain were analyzed for expression of the human λ2 gene in thymus, spleen, bone marrow, brain, liver, kidney, heart, and skeletal muscle tissue. Excised tissue was homogenized in guanidine isothiocyanate solution as described by Chirgwin et al. (supra), and total RNA was extracted for RNase protection analysis as described above. Each of the transgenic strains was found to express the human U266λ gene in all tissues assayed. Of the two λEμ strains, EG expressed λ mRNA at a higher level than did EH.

Susceptibility of Transgenic Mice to Infectious Diseases

The transgenic EG mouse line was originally established in a mouse colony where a large number of pathogens are endemic. These pathogens include Sendai virus, EDIM virus, mouse hepatitis virus, mycoplasma pulmonis, pinworm, and mites. The λEμ-EG founder animal, a CD-1 X FVB/N F1 male, who may have been a genetic mosaic, remained healthy and reliably sired litters that showed an autosomal inheritance pattern; however, very few of his transgenic male or female offspring born into the infected facility could successfully sire or raise litters. Furthermore, while the life expectancy of normal mice in this colony is more than two years, half of the hemizygous EG mice died from infectious diseases before they were 91 days old and no homozygous EG/EG mice survived more than one month. Under these conditions, the deaths appeared to be principally from acute pneumonia, probably due to a combination of Sendai virus and bacterial or mycoplasma infections. This high degree of susceptibility to infectious diseases suggests that these animals suffer from an immune defect, inherited as a dominant effect of the λEμ transgene. When the EG strain was derived to a specific pathogen-free facility by sterile cesarian section, none of the pathological conditions observed in mice raised in the infected facilities was evident. The EH mice and transgenic mice descended from the strains harboring the λ construct did not show unusual susceptibility to infectious diseases.

Examination of EG mice revealed that they had spleens that were 50% to 75% smaller than those of their wild-type litter mates; this difference persisted even when the mice were raised in a specific pathogen-free facility. The mice of all other transgenic strains had spleens which appeared normal.

Analysis of Lymphoid Cell Populations by Flow Cytometry

The B220 (Ly-5) cell surface antigen normally occurs only on murine B cells, including both early pre-B cells that have not yet rearranged their heavy chain genes and mature B cells (Kinkade et al., J. Immunol. 127:2262, 1981; McKeon and Rosenberg, Eur. J. Immunol. 15:295, 1985; and Cooper et al., Nature 321:616, 1986). As pre-B cells of wild-type mice mature, they begin to express low levels of surface heavy chain $\mu$. As maturation continues, B cells migrate to the spleen and begin strongly to express surface Ig.

Surface antigens expressed by the lymphoid cell population of $\lambda E\mu$ transgenic mice and wild-type mice were analyzed by flow cytometry using fluorescence-tagged antisera. Spleen suspensions in Hank's balanced salt solution (HBSS) were prepared by teasing apart spleens with forceps. Bone marrow cell suspensions were prepared by flushing femurs and tibias with HBSS using a syringe fitted with a 26 gauge needle. The single-cell suspensions were labeled with 1) rat anti-mouse B220 antibody (6B2, Coffman et al., Immunological Reviews 69:5, 1982) and fluorescein-conjugated goat anti-rat antibodies (Kirkegaard and Perry Laboratories, Gaithersberg, MD), 2) fluorescein-conjugated rat anti-mouse $\kappa$ (MRCOX20, Bioproducts for Science, Inc., Indianapolis, IN), or 3) fluorescein-conjugated goat anti-mouse $\mu$ (Southern Biotechnology Associates, Inc., Birmingham, AL). The labeled cells were analyzed on a Cytofluorograf IIS flow cytometer (Ortho Diagnostic Systems Inc., Westwood, MA).

EG and EH mice were found to have a consistently lower percentage of surface B220 (Ly-5)-positive cells in the spleen and bone marrow than found in wild-type mice (Table 1), implying that these mice are B cell deficient. These transgenic strains of mice also had a lower percentage of murine $\kappa$ chain-positive and murine $\mu$ chain-

positive spleen cells than did wild-type mice (Table 1).

Table 1:
Percentage of B cell Surface Antigen-Positive Lymphocytes

| Tissue Antigen | inbred FVB/N | STRAIN EG | EH | EGxC57BL/6 |
|---|---|---|---|---|
| Spleen | | | | |
| B220 | 39.9 ±7.1 | 2.1 ±0.97 | 8.7 | 10.9 ±1.4 |
| mouse κ | 36.9 ±11.1 | 2.3 ±2.0 | 7.8 | 7.4 ±0.1 |
| mouse μ | 39.3 ±10.8 | 1.6 ±0.93 | 7.9 | 12.7 ±1.5 |
| Bone Marrow | | | | |
| B220 | 27.2 ±2.4 | 6.5 ±1.7 | 26.4 | nd |

The two λEμ transgenic mice strains differ from each other in that the percentage of B220 (Ly-5)-positive cells in EG mice was found to be lower than that in EH mice (Table 1). Taken with the fact that EG mice have a higher level of mRNA expression than EH mice and are more severely immunodeficient, these results suggest that the degree of B cell deficiency in λEμ transgenic mice is dependent on the level of λ transgene expression, which may be related to transgene copy number, or the site of chromosomal integration. The transgenic offspring of a cross between an EG mouse and a C57BL/6 mouse were also analyzed.

Antibody staining to detect surface human λ revealed that spleen cells from EG mice lacked surface human λ despite the fact that the cells expressed human

mRNA. In contrast to their apparent B cell deficiency, EG mice appeared to have normal levels of T cells when assessed by the criteria of staining for CD3, CD4 and CD8 surface markers.

As a further measure of the lymphoid cell population in EG mice, cultured splenocytes were tested for uptake of tritiated thymidine (measured in 'cpm/$10^4$ cells' in a fixed time period) in response to concanavalin-A, a non-specific T cell mitogen, and lipopolysaccharide, a B cell mitogen (Anderson et al., Eur. J. Immunol. 2:349, 1972; Janossy et al., Clin. Exp. Immunol. 14:581, 1973). Lipopolysaccharide-induced uptake by EG splenocytes was only one-tenth the uptake observed for splenocytes from wild-type mice, while concanavalin-A-induced uptake by EG splenocytes was identical to uptake by wild-type splenocytes. These results provide additional evidence that the EG mice are severely B cell deficient, while suggesting that they have functional T cells.

Approximately 80-95% of the splenocytes of wild-type mice can be labelled with B cell- or T cell-specific markers. In contrast, only about 50% to 70% of the splenocytes of EG mice could be labeled with such markers. The remaining unlabeled cells bore surprisingly large quantities of the Sca-1 antigen as detected by the Ly-6A.2 antibody (van de Run et al., Proc. Natl. Acad. Sci. USA 86:4634, 1986; Aihara, et al., Eur. J. Immunol. 16:1391, 1986) in a fluorescence activated cell sorting assay (FACS assay). Normally, 10% of splenic lymphocytes are Sca-1-positive, and only weakly so; in contrast, 40% of the splenocytes of EG mice were strongly positive. The results of a FACS assay are depicted in Fig. 8 (The dashed line represents splenic lymphocytes from an EG mouse; the dotted line represents splenic lymphocytes from wild-type inbred FVB/N mouse; the solid line is a no

antibody control.). These strongly Sca-1-positive cells may represent an accumulation of B lineage cells behind the developmental block caused by the transgene, or they may be cells of the B lineage or other lineages that have proliferated in response to lymphokines secreted as a result of the lack of mature B cells. Alternatively, the Scal bearing cells may be abnormal cells sidetracked from the B cell lineages a result of transgene expression.

In Vitro Culture of EG Bone Marrow and Fetal Liver Cells

Bone marrow cells from wild-type mice can be grown into adherent feeder layers which support the proliferation of wild-type B cell lineage lymphocytes from adult bone marrow or fetal liver (Whitlock, et al., J. Immunologic. Meth. 67:353, 1984). While feeder layers established from EG bone marrow supported the long-term growth of wild-type, feeder-dependent, B cell lines derived from wild-type bone marrow or fetal liver cells, the converse was not true: bone marrow or fetal liver cells from EG mice would not establish long term cultures on feeder layers derived from bone marrow of wild-type mice. Thus, the bone marrow stroma of EG mice is capable of supporting B cell growth, but the their B cell precursors are developmentally blocked or die early in development. This is further evidence that the transgene causes a specific block in the development of B lineage cells.

Abelson Murine Leukemia Virus Transformation of EG Bone Marrow Cells and Fetal Liver Cells.

A quantitative A-MuLV lymphoid cell transformation assay, using the method of Rosenberg et al. (J. of Exp. Med. 143:1453, 1976), can be used to measure the number of B cell precursors present in fetal liver and bone marrow. The rate of A-MuLV transformation of EG embryonic 16 day fetal liver cells was about fifty-fold lower than for wild-type fetal liver cells, implying that

EG mice have a sharply lower number of B cell precursors. In several attempts, A-MuLV failed to transform EG bone marrow cells at all. These results provide further evidence that EG mice are B cell deficient.

**Use**

The animals of the invention can be used as models of human diseases characterized by B cell deficiency or defects in B cell function, and thus would be useful to develop diagnostic assays and to test potential therapeutics. For example, X-linked agammaglobulinemia is an inherited disorder characterized by a nearly complete absence of serum immunoglobulin, resulting in an unusual susceptibility to infection; examination of the lymphocyte populations of afflicted individuals reveals a severe deficiency of mature B cells, although pre-B cells are present in normal numbers. The animals of the invention might also be used to study a group of disorders referred to as common variable immunodeficiency (CVID). Individuals with CVID usually have a normal B cell count, but their B cells do not function normally. There are multiple causes of CVID; the underlying defects include inability to secrete immunoglobulins, absence of helper T cells (which are required for normal B cell function), B cells that do not respond to stimulatory signals, and the presence of auto-antibodies to B cells. The animals of the invention can be used to model various forms of CVID by transplantation with particular B cell populations. In addition, there are a number of diseases characterized by selective deficiency in a particular class of immunoglobulin, and the animals of this invention can be used to create models of these disorders.

Besides their utility as disease models, the B cell-deficient animals of this invention can be used to

examine the role of humoral immunity in any of several extremely complicated immunological processes characterized by intricate interactions between B cells and T cells, including antigen presentation, allergy, autoimmune disorders and graft rejection.

Because bone marrow derived from the transgenic mice of the invention is essentially devoid of B cell precursors, these bone marrow cells could be used as a feeder layer for growth of feeder-dependent B cells without risk of contamination by B cells endogenous to the feeder layer bone marrow.

Furthermore, the developmentally-blocked hematopoietic progenitor cells of these animals provide a source of material for study of the biochemistry and cell biology of B cell development. The absence of B cells in these mice very likely results in elevated levels of growth factors, and thus they may provide a source of novel lymphokines.

## Deposit

Embryos generated by crossing an EG mouse with a inbred FVB/N mouse have been deposited with the American Type Culture Collection on August 28, 1990 and bear the accession number ATCC No. 72003. Applicants' assignee, the President and Fellows of Harvard University, acknowledges its responsibility to replace these embryos should they die before the end of the term of a patent issued hereon, and its responsibility to notify the ATCC of the issuance of such a patent, at which time the deposit will be made available to the public. Prior to that time the deposit will be made available to the Commissioner of Patents under the terms of 37 CFR §1.14 and 35 USC §112.

## Other Embodiments

Other embodiments are within the following claims. For example, any rearranged λ gene derived from a human or any other non-mouse animal could be substituted for the human U266λ gene utilized in the example described above. The U266λ gene could be replaced by any DNA sequence that encodes the U266λ protein. Also included are transgenes that encode a protein substantially similar to the U266λ protein in that introduction of the transgene into a suitable animal will lead to the phenotype disclosed herein. Such proteins would include proteins that differ from the protein encoded by the U266λ gene in that they have conservative amino acid changes (e.g., one acidic amino acid is changed to another acidic amino acid), or deletions or insertions of non-essential amino acids. The enhancer region could be any natural or synthetic DNA sequence that induces a higher level of expression of the transgene, including viral enhancers, eukaryotic enhancers, or sequences that are capable of binding endogenous transcriptional activators, all of which are well known to those of ordinary skill in the art of eukaryotic gene expression. Also included in this invention are hematopoietic cells (such as B cell precursors) derived from a transgenic mouse of this invention.

We claim:

1. A transgenic FVB/N mouse bearing a transgene comprising a DNA sequence encoding an immunoglobulin λ light chain endogenous to a non-mouse animal.

2. The transgenic FVB/N mouse of claim 1, wherein said non-mouse animal is an animal in which the proportion of its immunoglobulin light chains that are immunoglobulin λ light chains is higher than the proportion typically found in FVB/N mice.

3. The transgenic FVB/N mouse of claim 2, wherein said animal is a human.

4. The transgenic FVB/N mouse of claim 3, wherein the constant region of said immunoglobulin λ light chain is selected from the λ1, λ2, λ3, and λ7 constant regions.

5. The transgenic FVB/N mouse of claim 4, wherein said constant region is the λ2 constant region.

6. The transgenic FVB/N mouse of claim 5, wherein said immunoglobulin λ light chain comprises the U266λ protein.

7. The transgenic FVB/N mouse of claim 1, wherein said transgene further comprises an enhancer region capable of enhancing expression of said DNA sequence.

8. The transgenic FVB/N mouse of claim 7, wherein said enhancer region is that of an immunoglobulin heavy chain gene.

9. The transgenic FVB/N mouse of claim 8, wherein said enhancer region is that of a mouse immunoglobulin heavy chain gene.

10. The transgenic FVB/N mouse of claim 9, wherein said enhancer region comprises the entire 995 bp XbaI-EcoRI fragment of the mouse immunoglobulin heavy chain gene.

11. The transgenic FVB/N mouse of claim 10, wherein said enhancer region comprises a portion of the 995 bp XbaI-EcoRI fragment sufficient to enhance expression of said DNA sequence.

12. The transgenic FVB/N mouse of claim 1, wherein mature B cells account for less than 10% of the splenic lymphocytes of said transgenic FVB/N mouse.

13. The transgenic FVB/N mouse of claim 1, wherein said transgenic FVB/N mouse is more susceptible to infectious disease than is a typical FVB/N mouse.

14. The transgenic FVB/N mouse of claim 1, wherein said transgenic FVB/N mouse has at least three times as high a percentage of splenic lymphocytes which bear the Sca-1 surface antigen as a typical FVB/N mouse.

15. The transgenic FVB/N mouse of claim 1, wherein fewer than 4% of the splenic lymphocytes of said transgenic FVB/N mouse bear the B220 (Ly-5) antigen.

16. A hematopoietic cell derived from the transgenic FVB/N mouse of claim 1.

17. A transgenic mouse derived from an embryo deposited as ATCC No. 72003, or a transgenic descendent of said mouse.

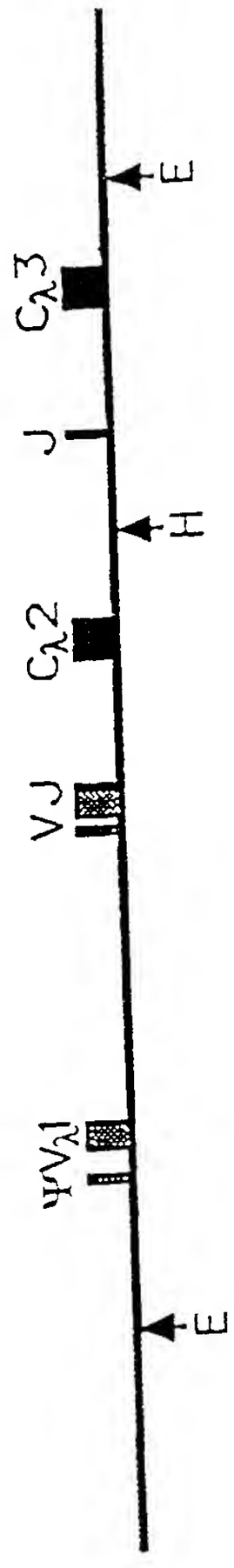



FIGURE 1

FIGURE 2 (sheet 1 of 5)

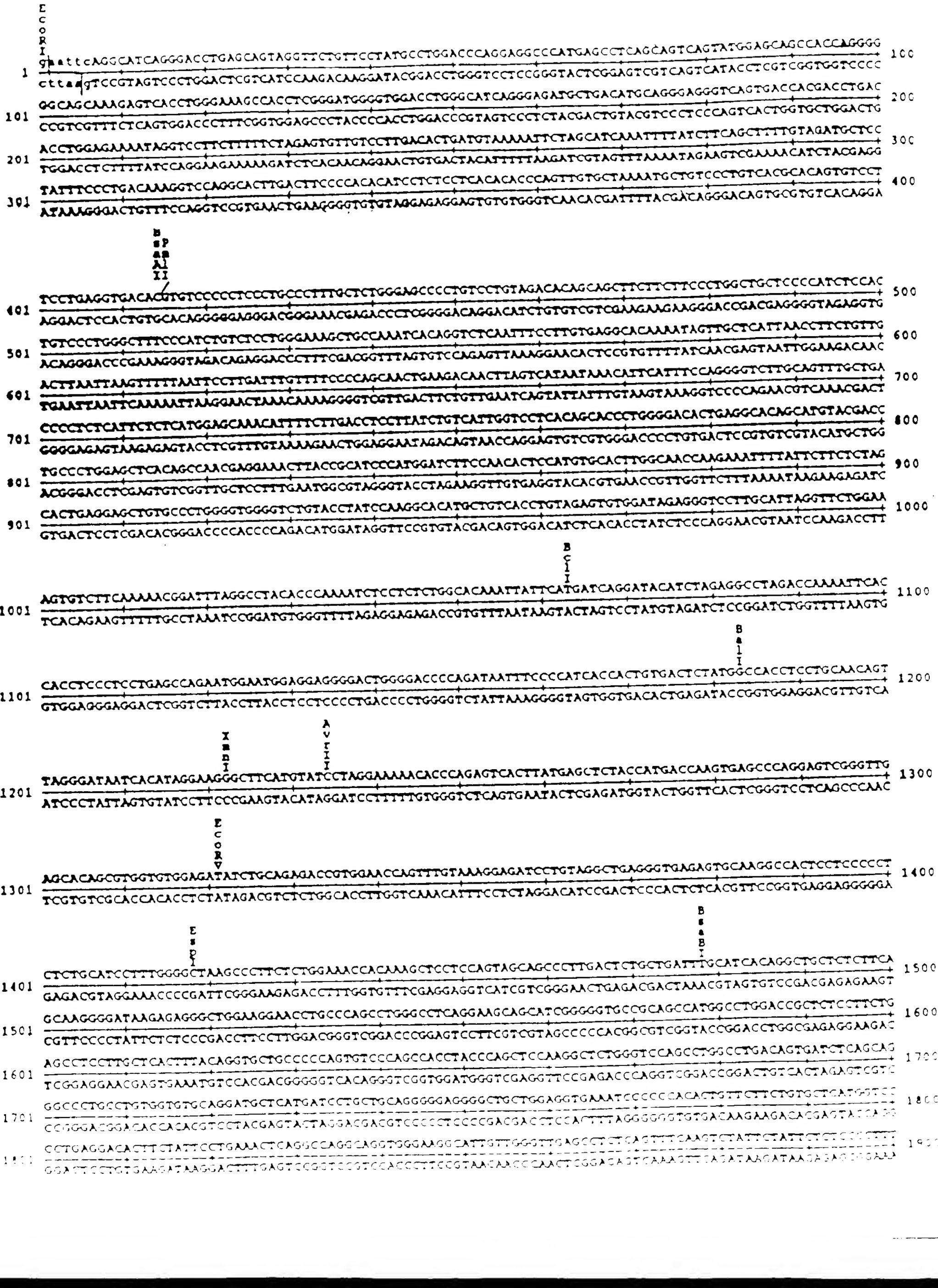
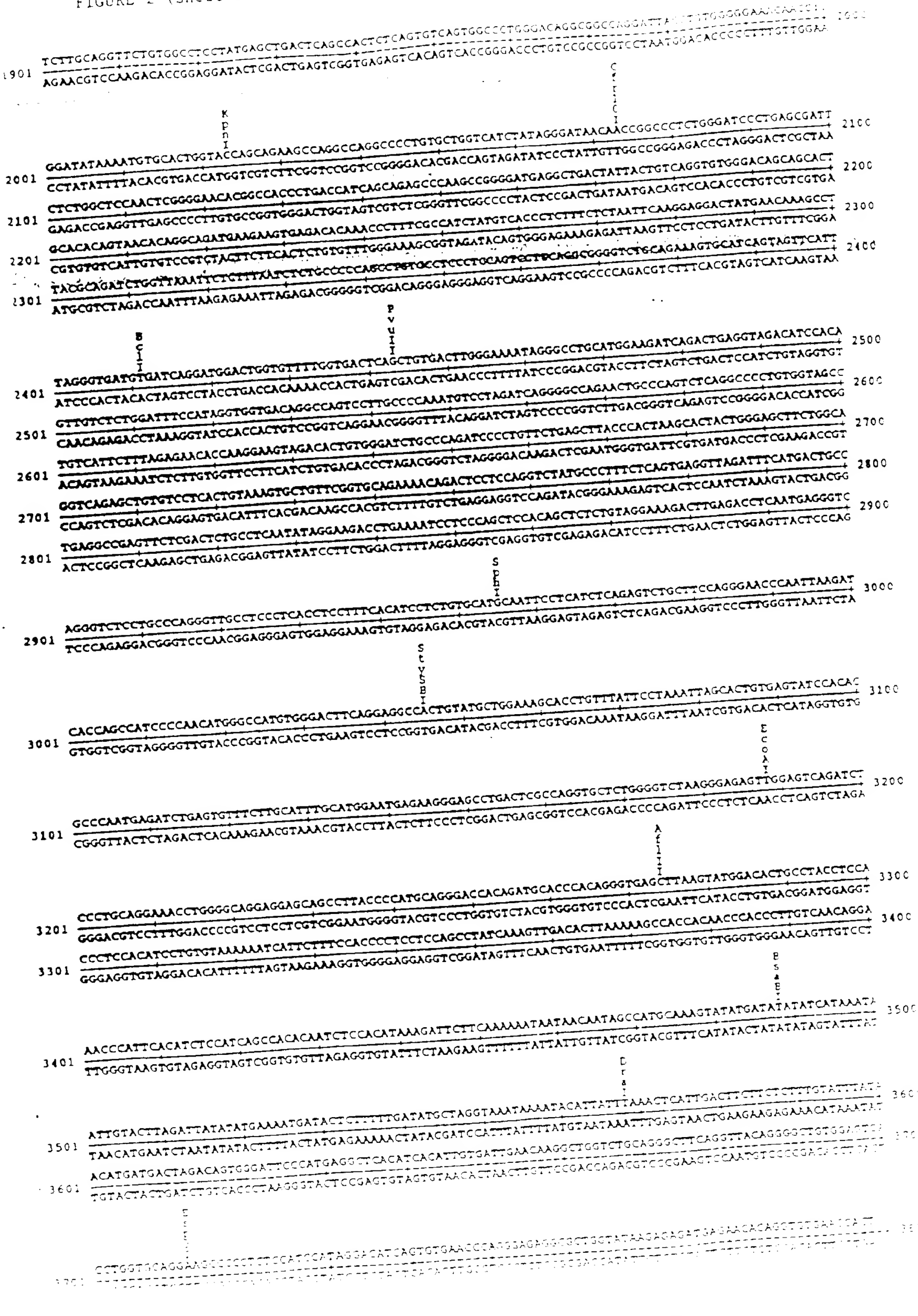


FIGURE 2 (sheet 2 of



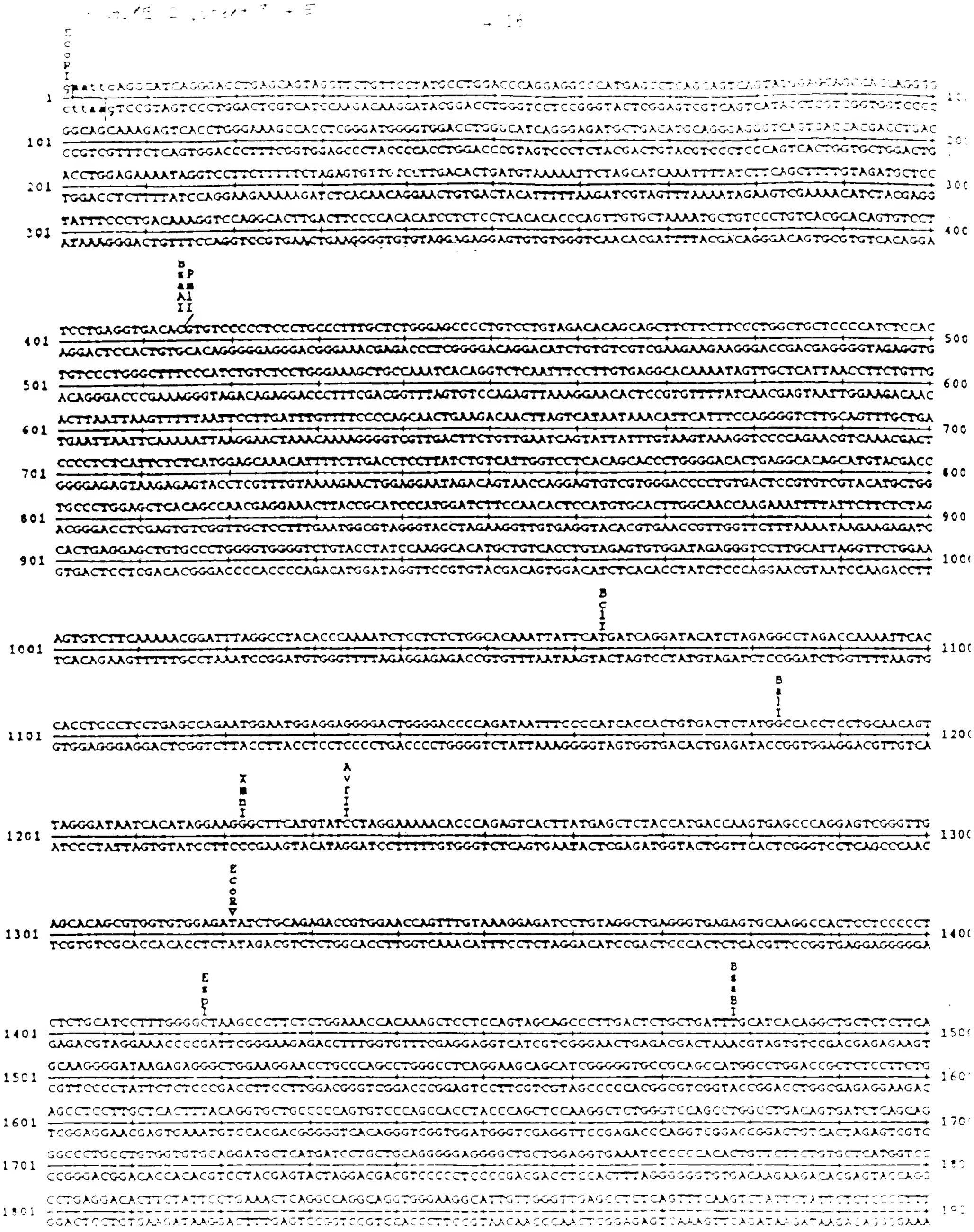

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FIGURE 2 (sheet 4 of 5)

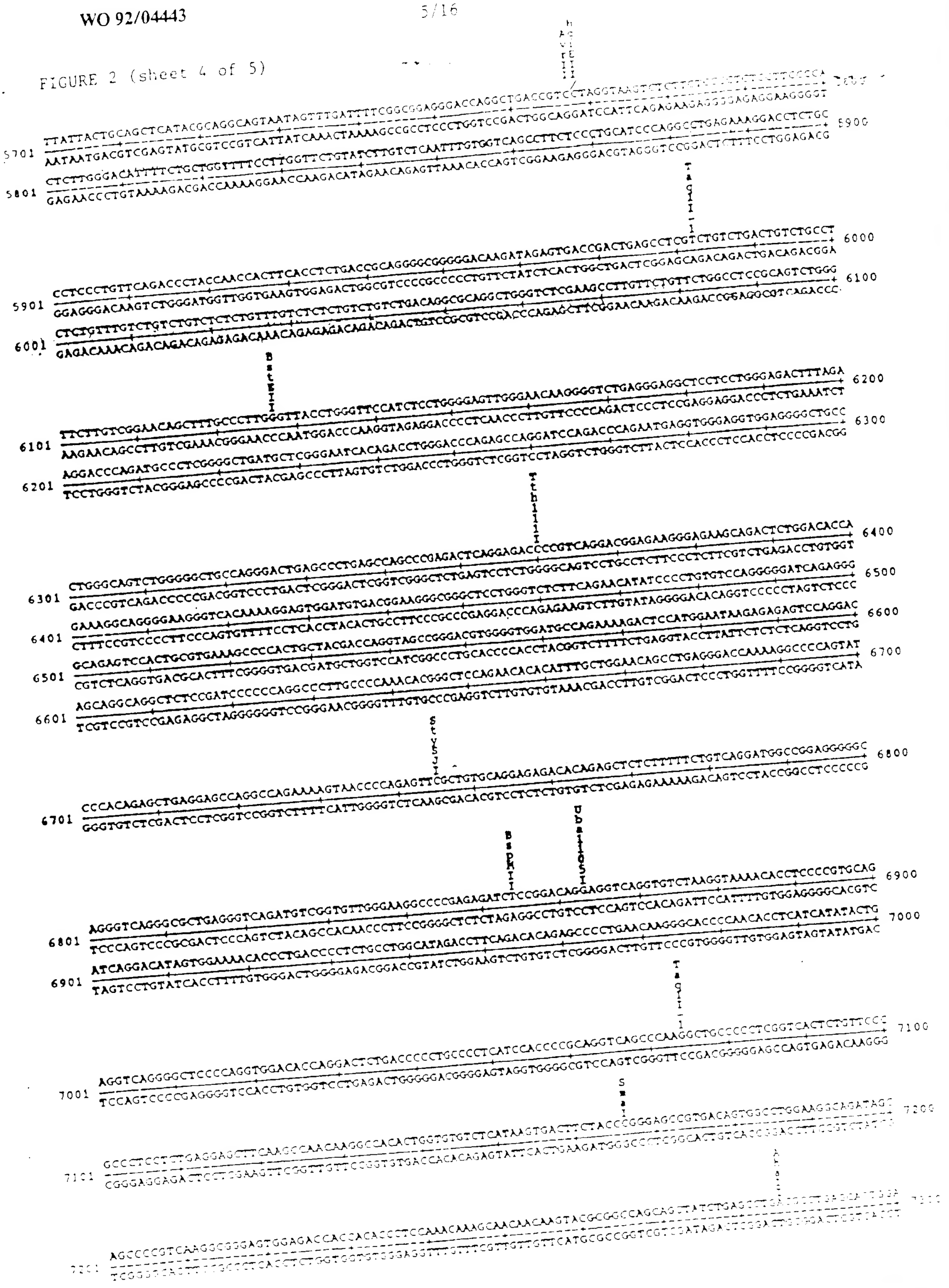
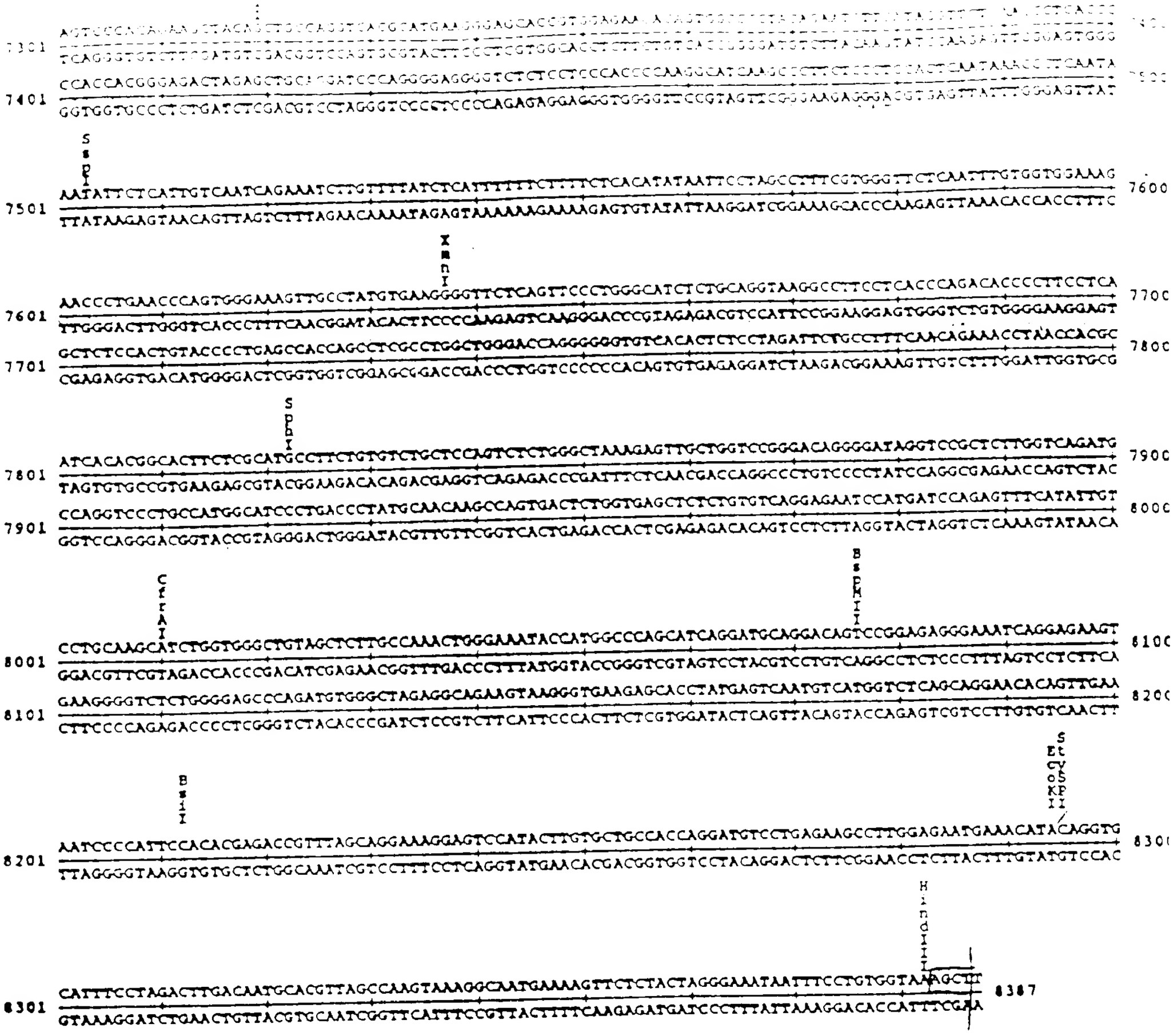


FIGURE 2
Sheet 5 of 5



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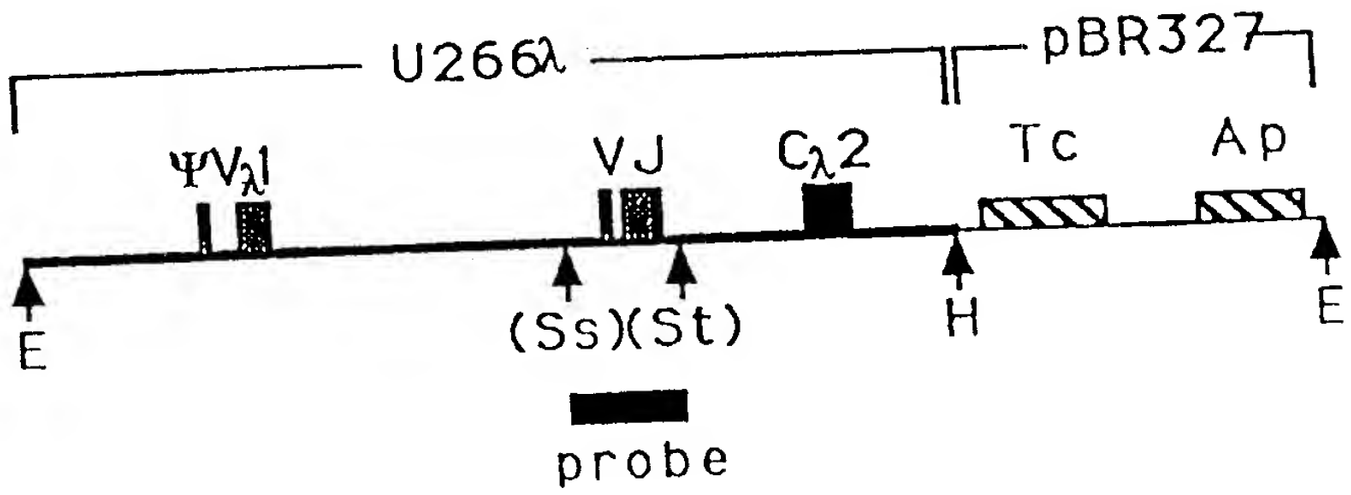

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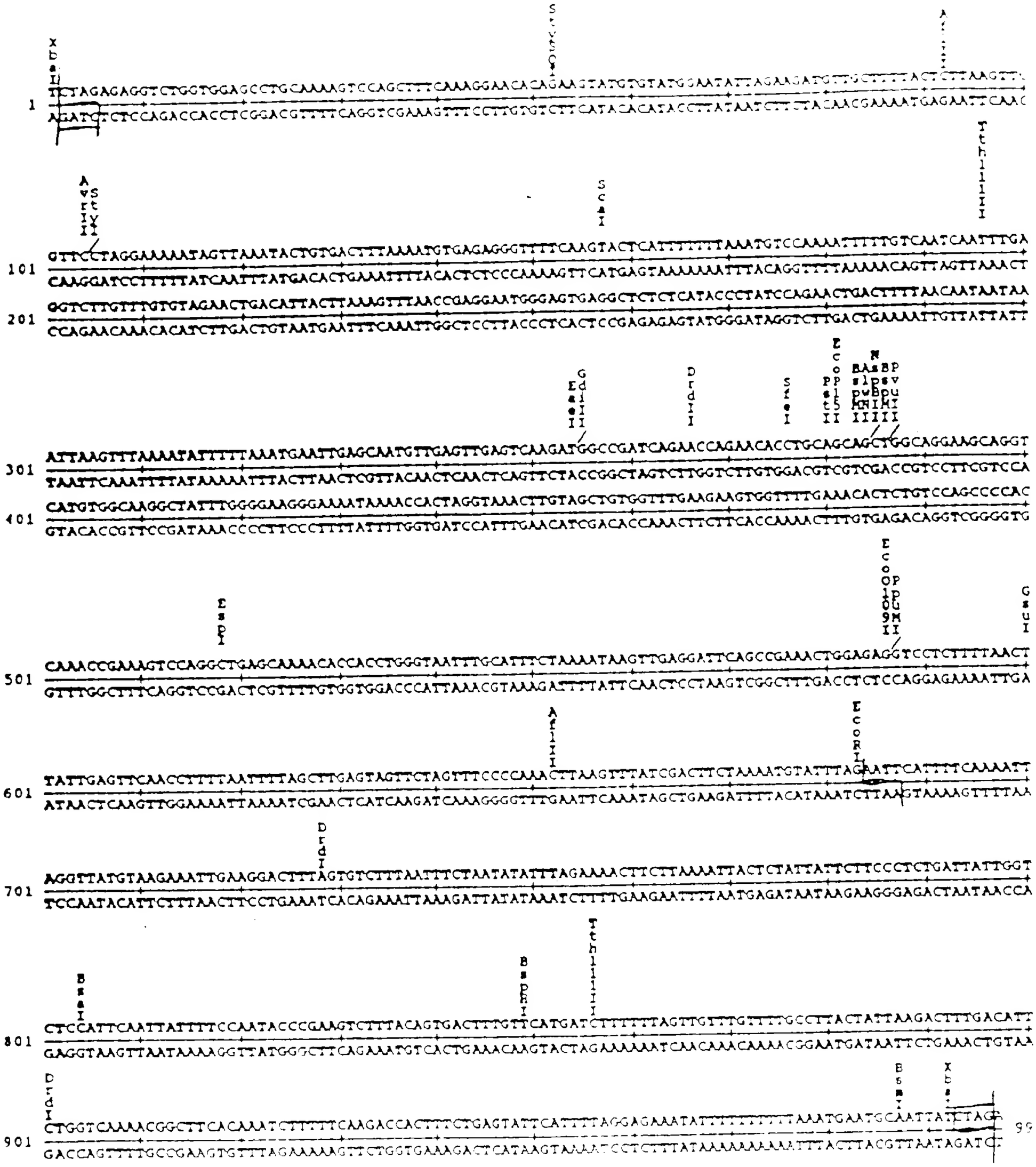
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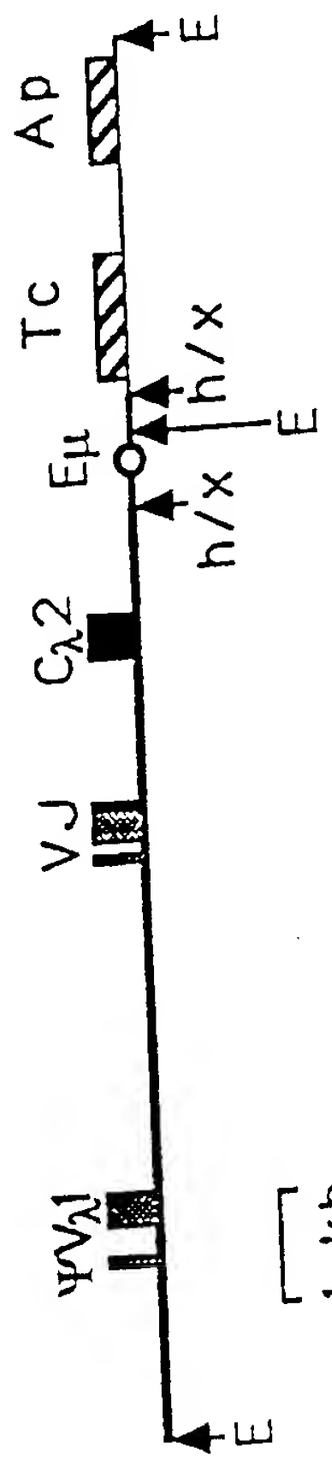


FIGURE 5

FIG. 4B 7 (sheet 2 of 5

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FIGURE 7
(sheet 3 of 5)

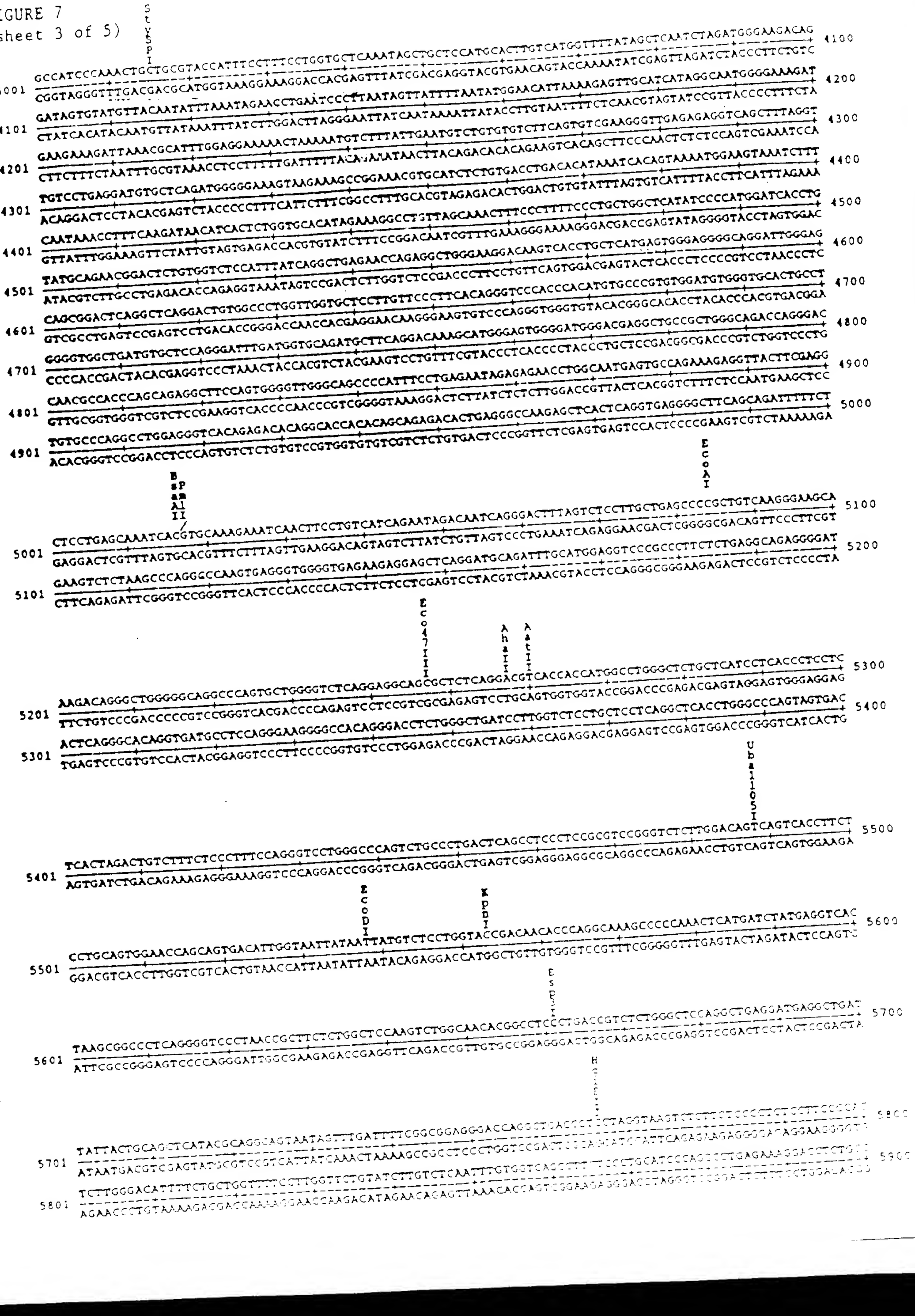


FIGURE 7 (sheet 4 of

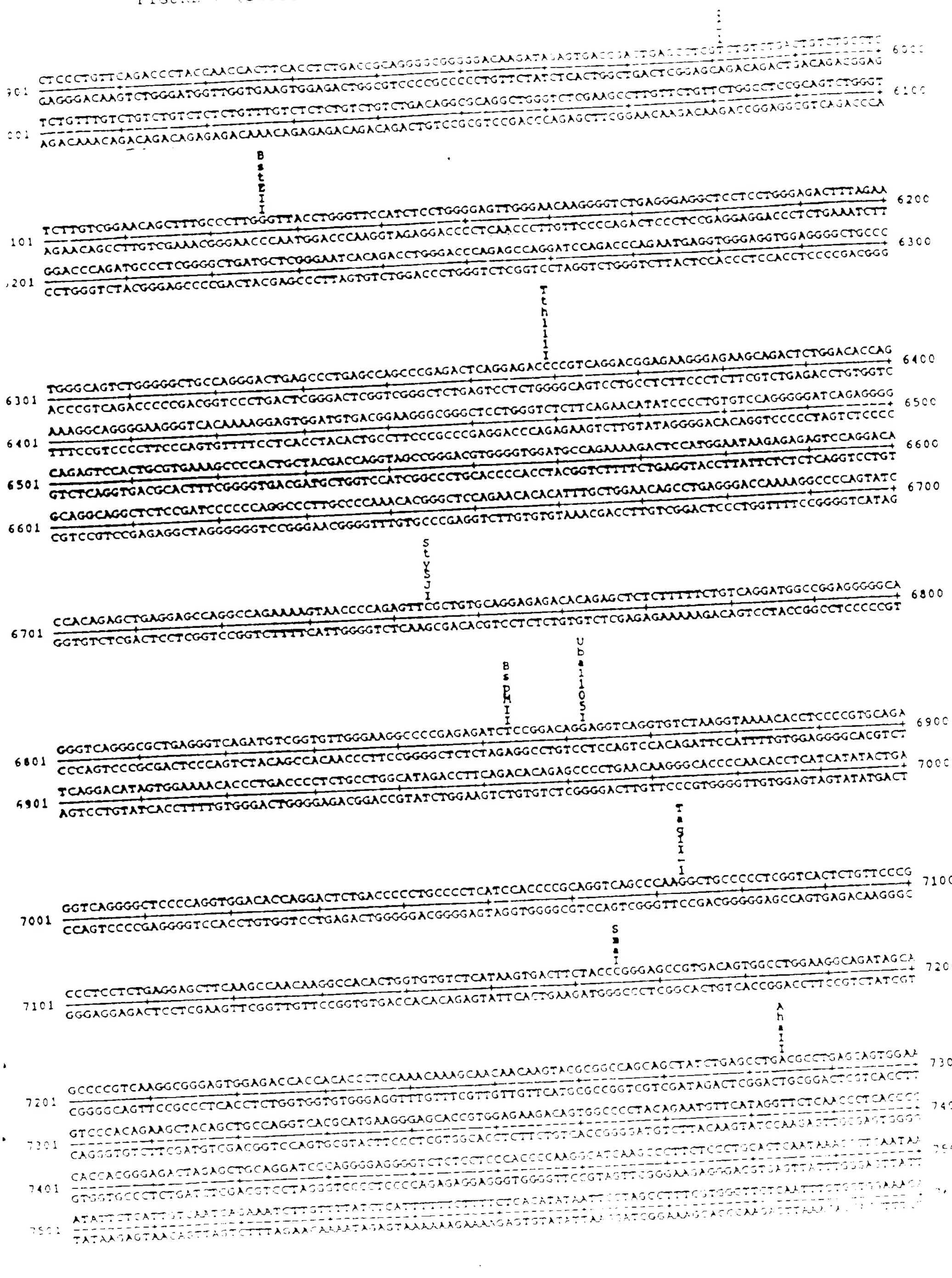


FIGURE 7 (sheet 5 of 5)

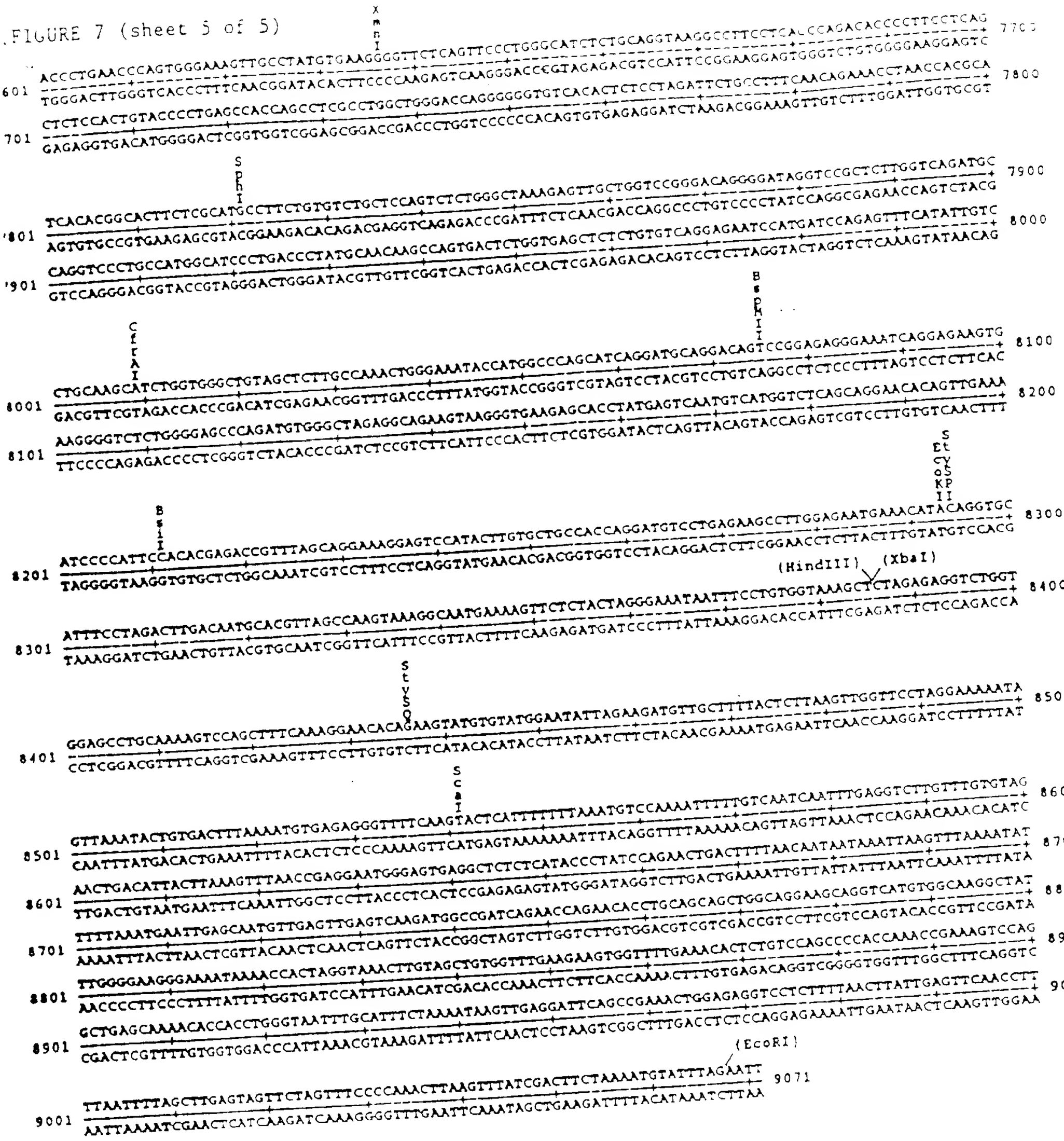
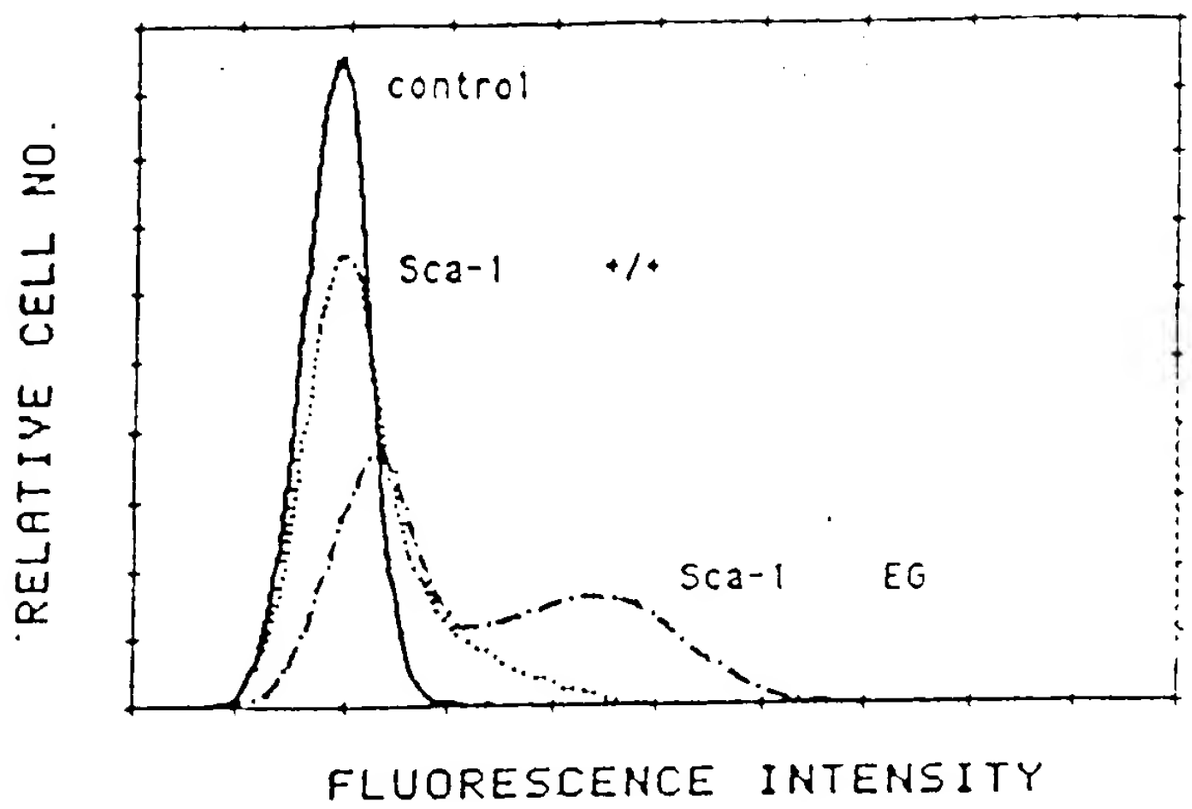


FIGURE 8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